

Data Evaluation Report on the Toxicity of BAS 500F to Fish, Early Life Cycle

PMRA Submission Number {.....}

EPA MRID Number 454201-01

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D275457
	OECD Data Point	{.....}
	EPA MRID	454201-01
	EPA Guideline	§ 72-4a

Test material: BAS 500 F

Purity: 93.5%

Common name Pyraclostrobin
chemical name: Methyl-*N* [[[1-(4-chlorophenyl) pyrazol-3-yl] oxy] *o*-tolyl] *N*-methoxycarbamate
IUPAC Methyl *N*-(2-{[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxymethyl} phenyl) *N*-methoxy carbamate
CAS name Methyl [2-[[[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl] oxy] methyl] phenyl] methoxycarbamate
CAS No. 175013-18-0
synonyms
EPA PC Code: 099100
Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]

Primary Reviewer(s): Santhini Ramasamy, Ph.D., M.P.H., D.A.B.T.
Environmental Scientist, OPP/EFED/ERB I

Santhini Ramasamy
2-26-02

Date: 6-27-01

Secondary Reviewer(s): Regi Mathew, Ph.D., Scientific Evaluation Officer, EAD, PMRA

Regi Mathew
3-01-02

Date: {.....}

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Boeri, R.L., D. C. Wyskiel, T. J. Ward and C.M. Holmes. 2001. Early Life Stage Toxicity of BAS 500F to the Sheepshead Minnow, *Cyprinodon variegatus*. Laboratory: T.R. Wilbury Laboratories, Inc., 40 Doaks Lane, Marblehead, Massachusetts 01945, Report Number: 2126-BA. Sponsor: BASF Corporation, Agricultural Products Group, P.O. Box 13528, Research Triangle Park, NC 27709-3528, Study No: 64548, May 25, 2001.



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EXECUTIVE SUMMARY:

The 36-day chronic toxicity of pyraclostrobin to early life stage of sheepshead minnow (*Cyprinodon variegatus*) was studied under flow through conditions. Less than 24 hours old fertilized embryos (80/group in four replicates) were exposed to control, solvent control, and test chemical mean measured concentrations of 2.91, 5.57, 10.8, 24.0, and 44.5 µg a.i./L. The test system was maintained at 29 to 30.8°C and a pH of 7.5 to 8.1 **The 36-day NOEC and LOEC values, based on survival effects, were 10.8 and 24 µg a.i./L, respectively.** There were no significant changes in time to hatch, sublethal effects and growth parameters such as length and dry weight of young fish. However, a significant increase in wet weight of young fish was observed in all test groups, but this effect was not considered for deriving toxicity values. The sensitive end points were survival effects during hatch (day 4) and post-hatch (day 36) periods.

This study is scientifically valid and classified as **CORE**.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: EPA Subdivision E- § 72-4(a) and ASTM, 1998 Standards were followed.
No major deviations from the guidelines were observed.

COMPLIANCE: GLP compliance as regulated under FIFRA 40 CFR Part 160. Signed and dated GLP and Quality Assurance statements were provided.

A. MATERIALS:

1. Test Material BAS 500F

Description: Brown Solid

Lot No./Batch No. : N68

Purity: 93.5 % a.i.

Stability of Compound: Relatively stable under test conditions
(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of

test chemicals:

Test substance was stored at room temperature in the dark

2. Test organism:

Species: Sheepshead minnow (*Cyprinodon variegatus*)

Age /embryonic stage at test initiation: Less than 24 hours old

(EPA requires fish embryos 2 to 24 hours old.)

Method of collection of the fertilized eggs: Collected during natural spawning of up to 28 pairs of conditioned adult fish

Source: Aquatic BioSystems, Inc., Fort Collins, CO

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding study: A range-finding study was conducted for 7 days under the static-renewal conditions at the test concentrations of 0 (Control and Solvent Control), 1.0, 10, 50, 100, and 200 µg/L (10 organisms/group). The organisms survived 100 % in both the controls and at two lower concentrations (≤ 10 µg/L). Zero percent mortality was observed in higher concentrations (≥ 50 µg/L). Fish exposed to 50 µg/L BAS 500 F exhibited erratic swimming on day 4 and immobility on day 5.

b. Definitive Study: A definitive study was conducted for 36 days under flow-through conditions at the nominal concentrations of 0 (Control and Solvent Control), 3.4, 6.5, 13, 25, and 50 µg/L (80 embryos/group; Following hatching, 40 organisms/group). The hatching success, time to hatch, post-hatch survival, sublethal effects and growth parameters were measured.

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Table 1 . Experimental Parameters

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Parameter	Details	Remarks
		Criteria
		<p>EPA requires that solvent should not exceed 0.1 ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.</p> <p>OECD requires that solvent must have no effect on survival nor produce any other adverse effects; concentration should not be greater than 0.1 ml/L.</p>
Number of replicates		Acceptable
control:	4	
solvent control:	4	EPA requires 4 replicates per concentration
treated ones:	4	EPA/OECD require solvent control when a solubilizing agent has been used.
Test condition:		Acceptable
static renewal/flow through:	Flow through system	
type of dilution system for flow through method:	The dilution system constructed at T.R. Wilbury Laboratories was used. No other mixing chamber was used. The volume of media delivered to a replicate test vessel was within 10% of the other replicate.	
flow rate:	6.7 volume additions/24 hours	Intermittent flow proportional diluters or continuous flow serial diluters should be used. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.
renewal rate for static renewal:	N/A	<p>Toxicant Mixing:</p> <ol style="list-style-type: none"> 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10%.
Aeration, if any	Dilution water was aerated prior to entering the proportional diluter system. Test tanks/embryo cups were not aerated.	Acceptable
		Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.
Duration of the test		

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Parameter	Details	Remarks
		Criteria
		<i>EPA requires 32 days</i>
Embryo cups, if used type/material: (glass/stainless steel) size: fill volume:	Glass cylinders (8.5 cm diameter) fitted on one end with 350 micron Nitex screen were suspended in the test vessels. Fill volume not reported. The cylinders were rocked at approximately 3 rpm to ensure adequate flow of media around the embryos.	Acceptable
		<i>EPA requires 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.</i>
Test vessel type/material: (glass/stainless steel) size: fill volume:	Glass aquaria (9 L; 15x30x20 cm) were used. Each aquarium maintained a constant volume of approximately 7 L (16-cm depth).	Acceptable
		<i>EPA/OECD requires all glass or glass with stainless steel frame.</i>
source of dilution water	The dilution water was natural seawater collected from the Atlantic ocean at T.R. Wilbury Laboratories. The salinity was adjusted to 15 to 16 parts per thousand by the addition of deionized water stored in polyethylene tanks where it was aerated and recirculated through particle filters, activated carbon, and a UV sterilizer.	Acceptable
		<i>EPA requires natural or reconstituted water; natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants. For Saltwater fish the salinity should be greater than or equal to 15 part per thousand. OECD accepts any water in which the test species show control survival at least as good as presented in SEP.</i>

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Parameter	Details	Remarks
		Criteria
<p>Water parameters:</p> <p>Hardness:</p> <p>pH:</p> <p>Dissolved oxygen:</p> <p>Temperature (s) (record all the temperatures used for different life stages):</p> <p>photoperiod:</p> <p>salinity (for marine or estuarine species):</p> <p>other measurements:</p> <p>interval of water quality measurements:</p>	<p>Hardness not reported</p> <p>7.5-8.1</p> <p>5.4-7.9 mg/L</p> <p>29-30.8°C</p> <p>16 hours light/8 hours darkness</p> <p>15-16 parts per thousand</p> <p>None</p> <p>Weekly ranges for dissolved oxygen, pH, temperature, salinity were reported for each replicate aquaria.</p> <p>Dissolved oxygen concentration was greater than 75% saturation.</p>	<p>Weekly ranges were reported in each replicate per treatment for dissolved oxygen, salinity, temperature and pH. The more specific intervals were not mentioned.</p> <p>Acceptable</p> <hr/> <p><i>EPA requires hardness of 40 to 48 mg/L as CaCO₃ and pH of 7.2 to 7.6 is recommended. DO must be measured at each conc. at least once a week; freshwater parameters in a control and one concentration must be analyzed once a week.</i></p> <p><i>Temperature depends upon test species; should not deviate by more than 2°C from appropriate temperature.</i></p> <p><i>OECD requires DO concentration between 60 - 90% saturation. As a minimum DO, salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test. Temperature should be measured continuously.</i></p>
<p>Post-hatch details:</p> <p>when the post-hatch period began:</p> <p>number of hatched eggs (alevins)/ treatment released to the test chamber on what day, the alevins were released from the incubation cups to the test chamber</p>	<p>Hatch began on day 3 for most of the groups. For the two highest dose groups the hatching began on day 4. The percent hatch in the controls ranged 95-100%. The hatched eggs were thinned to 40 from 80 embryos per treatment on day 4 or 5 for post-hatching studies.</p>	<p>Acceptable</p> <hr/> <p><i>EPA requires % of embryos that produce live fry must be > 50% in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.</i></p>

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Parameter	Details	Remarks
		Criteria
Post-hatch Feeding: start date: type/source of feed: amount given: frequency of feeding:	Beginning on Day 6, the fish were offered newly hatched <i>Artemia salina</i> nauplii, <i>ad libitum</i> , two or three times daily. Feeding was continued until 1 day before study termination (Day 36).	Acceptable
Stability of chemical in the test system	Stable under the conditions tested.	Acceptable
Recovery of chemical: Frequency of measurement: LOD: LOQ:	83-96% of the nominal concentrations. Samples were collected from alternate replicates in weekly intervals 0.234 ng-1.15 ng depending on the column (HP 1050 or HP 1100) 0.5 µg/L	Acceptable
Positive control {if used, indicate the chemical and concentrations}	None	N/A
Fertilization success study, if any number of eggs used: on what day the eggs were removed to check the embryonic development:	Eggs collected from 28 pairs of conditioned adult fish were shipped overnight to the testing lab. Fertilization was confirmed in the testing lab visually at test initiation as less than 24 hours post fertilization.	Acceptable
Other parameters, if any	N/A	N/A

2. Observations:

Table 2: Observations

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Criteria	Details	Remarks/Criteria
Parameters measured including the sublethal effects/toxicity symptoms	Number of embryos hatched - Time to hatch - Daily survival during hatching and post-hatching - Sublethal effects - Measurement of growth (body weight and length) -evaluated statistically	Acceptable ----- <i>EPA minimally requires:</i> - Number of embryos hatched; - Time to hatch; - Mortality of embryos, larvae, and juveniles; - Time to swim-up (if approp.); - Measurement of growth; - Incidence of pathological or histological effects; - Observations of other effects or clinical signs.
Observation intervals/dates for: egg mortality: no. of eggs hatched: mortality of fry (e.g.alevins): swim-up behavior: growth measurements: embryonic development: other sublethal effects	Daily observations were reported for mortality of eggs, fry and sublethal effects Growth measurements were conducted at the end of the experimental period.	Acceptable -----
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes	
Other observations, if any	None	

II. RESULTS AND DISCUSSION**A. MORTALITY:**

Table 3: Effect of BAS 500 F on egg hatching and survival at different life stage of fish.

Treatment (µg a.i./L) Mean Measured Concentrations	Egg hatched/embryo viability			Time to hatch (days)	Number of Day 5 fish (post-thinning)	Juvenile-survival on day 36		¹ Growth-length (mm)	¹ Growth-wet weight (mg)	¹ Growth-dry weight (mg)
	No. of eggs at day 0	hatch/embryo viability (day 4)				No. dead	percent mortality			
		No.	%							
Control	80	80	100	3-4	40	38	95	17.7± 1.5	85.0 ±19.6	20.4 ± 4.2
Solvent control	80	76	95	3-4	40	40	100	18.1± 2.0	85.5 ±22.2	20.8 ± 5.1
2.91	80	78	98	3-4	40	38	95	18.5± 1.4	97.5 ±23.4	21.8 ± 4.5
5.57	80	79	99	3-4	40	38	95	19.0± 1.7	98.6 ±22.6	23.8 ± 4.5
10.8	80	79	99	3-4	40	39	98	18.6± 1.1	98.3 ±15.6	22.3 ± 2.6
24	80	23	29	4-5	40	22	55	18.7± 1.3	108.7 ± 21.2	23.2 ± 4.6
44.5	80	0	0	² 4-5	-	-	-	---		---
NOEC (µg a.i./L)	-	-	10.8	10.8	-	-	10.8	24	24	24
LOEC (µg a.i./L)	-	-	24	24	-	-	24	44.5	44.5	44.5
Positive control, if used	None	-	-	-	-	-	-	-		-

¹ Results represent mean of 40 individual fish per treatment instead of the mean of 4 individual replicate means. The mean values of replicate means are provided in Summary of Statistics attachment at the end of the DER.

²Hatched dead

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

Sublethal effects as fish exhibiting lethargy and/or erratic swimming were observed at 10.8 µg/L on day 4, and at 24.0 µg/L on days 4 and 5. No sublethal effects were observed at any concentrations during post-hatching.

C. REPORTED STATISTICS:

TOXSTAT 3.3 was used to analyze the data. Control data and solvent control data were compared using a parametric t' test and found not to be significantly different ($\alpha=0.05$) for post-hatch survival, total length, wet weight and dry weight. Chi-square test was used to determine if data were normally distributed, and Bartlett's test was used to determine if variances were homogeneous. A one-way ANOVA and Bonferroni's test, William's test or Kruskal and Wallis test was used to compare treatment and control means. Survival and sublethal effects were arc sine transformed prior to statistical analyses.

Table 4: Reported Statistics

Endpoint	Method	NOEC	LOEC
Hatch Survival	Chi-square, Bartlett's, ANOVA, and Bonferroni or Williams	10.8 ppb	24 ppb
Terminal Survival	Chi-square, Bartlett's, ANOVA, and Bonferroni or Williams	10.8 ppb	24 ppb
Time to hatch	Chi-square, Bartlett's, ANOVA, and Bonferroni or Williams	10.8 ppb	24 ppb
Wet Weight	Chi-square, Bartlett's, ANOVA, and Bonferroni or Williams	24 ppb	44.5 ppb
Dry Weight	Chi-square, Bartlett's, ANOVA, and Bonferroni or Williams	24 ppb	44.5 ppb
Length	Chi-square, Bartlett's, ANOVA, and Bonferroni or Williams	24 ppb	44.5 ppb

D. VERIFICATION OF STATISTICAL RESULTS:

Survival was assessed using Fisher's exact test. Continuous data (length, wet weight, and dry weight) were assessed by Williams test. Solvent control was used as baseline for all comparisons.

Statistical Method (Fisher's exact test):

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NOEC: 10.8 µg a.i./L (post-hatch survival)

LOEC: 24 µg a.i./L (post-hatch survival)

(Williams test):

NOEC: <2.91 µg a.i./L (wet weight)

LOEC: 2.91 µg a.i./L (wet weight)

Since the treatment groups had significantly higher wet weight than the solvent control, the NOEC/LOEC values based on wet weight were not considered in the conclusions.

E. STUDY DEFICIENCIES: No.

F. REVIEWER'S COMMENTS: Statistical analysis of the data suggest a significant increase in the wet weight of fish exposed to pyraclostrobin at ≥ 2.91 ppb. It is not known if there were abnormal histological changes in the tissues such as tumor. Similar trend was also observed in the dry weight and length measurements. However, these results were not statistically significant.

G. CONCLUSIONS: In a 36-day chronic early life stage toxicity study, sheepshead minnow (*Cyprinodon variegatus*) were exposed to mean measured concentrations of pyraclostrobin at 0 (control and solvent control), 2.91, 5.57, 10.8, 24.0, and 44.5 µg a.i./L under flow through conditions. There were no significant changes in time to hatch, sublethal effects and growth effects such as length, and dry weight. The sensitive end points were hatch and post-hatch survival at 36 days. **The 4-day as well as 36-day NOEC and LOEC values, based on survival effects, were 10.8 and 24 µg a.i./L, respectively.** The results are in agreement with study author's results. This study is classified as **CORE**.

III. REFERENCES: None

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Verification of Statistical Results**Pyraclostrobin -Post hatch Survival**

CRITICAL FISHER'S VALUE (40,40,40) (p=0.05) IS 35. b VALUE IS 22.

Since b is less than or equal to 35 there is a significant difference between CONTROL and TREATMENT at the 0.05 level.

SUMMARY OF FISHER'S EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	40	0	
1	2.91 ug	40	2	
2	5.57 ug	40	2	
3	10.8 ug	40	1	
4	24 ug	40	18	*

pyraclostrobin - length

length

File: sheepl

Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	solvent	4	17.900	18.400	18.100
2	2.91 ug	4	17.700	19.300	18.525
3	5.57 ug	4	18.300	19.500	19.000
4	10.8 ug	4	18.300	19.400	18.675
5	24 ug	4	17.900	19.900	18.875

length

File: sheepl

Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	solvent	0.047	0.216	0.108	1.19
2	2.91 ug	0.442	0.665	0.333	3.59
3	5.57 ug	0.253	0.503	0.252	2.65
4	10.8 ug	0.269	0.519	0.259	2.78
5	24 ug	1.042	1.021	0.511	5.41

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length

File: sheep1

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solvent	4	18.100	18.100	18.100
2	2.91 ug	4	18.525	18.525	18.525
3	5.57 ug	4	19.000	19.000	18.838
4	10.8 ug	4	18.675	18.675	18.838
5	24 ug	4	18.875	18.875	18.875

length

File: sheep1

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
solvent	18.100				
2.91 ug	18.525	0.938		1.75	k= 1, v=15
5.57 ug	18.838	1.627		1.84	k= 2, v=15
10.8 ug	18.838	1.627		1.87	k= 3, v=15
24 ug	18.875	1.710		1.88	k= 4, v=15

s = 0.641

Note: df used for table values are approximate when v > 20.

Pyraclostrobin-wet weight

File: sheep2

Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA

TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Sol Control	4	81.600	86.800	85.500
2	2.91 ppb	4	92.300	105.200	97.725
3	5.57 ppb	4	92.400	106.200	98.925
4	10.8 ppb	4	92.200	105.800	98.475
5	24 ppb	4	94.200	130.800	111.475

wet weight

File: sheep2

Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA

TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Sol Control	6.760	2.600	1.300	3.04
2	2.91 ppb	38.262	6.186	3.093	6.33
3	5.57 ppb	51.729	7.192	3.596	7.27

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4	10.8 ppb	35.876	5.990	2.995	6.08
5	24 ppb	319.863	17.885	8.942	16.04

wet weight

File: sheep2

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol Control	4	85.500	85.500	85.500
2	2.91 ppb	4	97.725	97.725	97.725
3	5.57 ppb	4	98.925	98.925	98.700
4	10.8 ppb	4	98.475	98.475	98.700
5	24 ppb	4	111.475	111.475	111.475

wet weight

File: sheep2

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Sol Control	85.500				
2.91 ppb	97.725	1.817	*	1.75	k= 1, v=15
5.57 ppb	98.700	1.962	*	1.84	k= 2, v=15
10.8 ppb	98.700	1.962	*	1.87	k= 3, v=15
24 ppb	111.475	3.861	*	1.88	k= 4, v=15

s = 9.513

Note: df used for table values are approximate when v > 20.

Pyraclostrobin - Dry weight

File: sheep3

Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Sol Control	4	20.200	21.200	20.775
2	2.91 ppb	4	20.200	24.200	21.825
3	5.57 ppb	4	22.600	24.800	23.850
4	10.8 ppb	4	21.400	22.900	22.300
5	24 ppb	4	19.000	27.600	23.500

Dry weight

File: sheep3

Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

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GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Sol Control	0.176	0.419	0.210	2.02
2	2.91 ppb	3.056	1.748	0.874	8.01
3	5.57 ppb	0.863	0.929	0.465	3.90
4	10.8 ppb	0.540	0.735	0.367	3.30
5	24 ppb	17.240	4.152	2.076	17.67

Dry weight

File: sheep3

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol Control	4	20.775	20.775	20.775
2	2.91 ppb	4	21.825	21.825	21.825
3	5.57 ppb	4	23.850	23.850	23.075
4	10.8 ppb	4	22.300	22.300	23.075
5	24 ppb	4	23.500	23.500	23.500

Dry weight

File: sheep3

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Sol Control	20.775				
2.91 ppb	21.825	0.710		1.75	k= 1, v=15
5.57 ppb	23.075	1.555		1.84	k= 2, v=15
10.8 ppb	23.075	1.555		1.87	k= 3, v=15
24 ppb	23.500	1.842		1.88	k= 4, v=15

s = 2.092

Note: df used for table values are approximate when v > 20.